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(54) **Anti-atherogenic agents.**

(57) As an anti-atherogenic agent, a substance or composition capable of lowering, in human plasma, the concentration of free sulfhydryl groups forming part of homocysteine and/or cysteine molecules, thereby inhibiting directly the formation of atherosclerotic plaques in the human body.

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THIS INVENTION relates to anti-atherogenic agents.

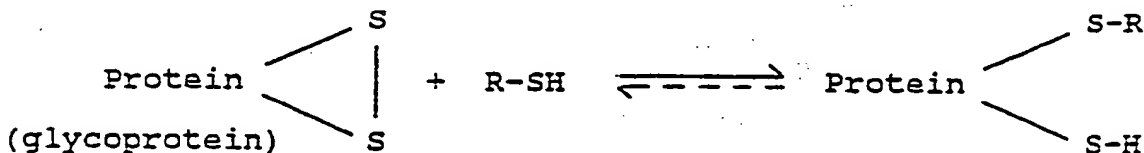
According to a first aspect of this invention, there is provided, as an anti-atherogenic agent, a substance or composition capable of lowering, in human plasma, the concentration of free sulfhydryl groups forming part of homocysteine and/or cysteine molecules, thereby inhibiting directly the formation of atherosclerotic plaques in the human body.

According to a second aspect of the invention, there is provided, as an anti-atherogenic agent, a substance or composition capable of inhibiting, ie hindering and/or lowering, the deleterious disulfide exchange reaction of homocysteine and/or cysteine with structural/functional proteins in arterial walls, thereby inhibiting directly the formation of atherosclerotic plaques in the human body.

The substance or composition may then comprise at least one compound capable of reacting directly or indirectly with the arterial wall cells and/or with the structural/functional proteins of the arterial wall cement, ie intercellular and/or cell-substrate cement, so as to prevent or hinder the homocysteine and/or cysteine molecule(s) from structurally damaging the group of structural/functional proteins such as fibronectin, in general known as glycoproteins, thereby to reduce directly or indirectly the mechanical and/or chemical stress on susceptible disulfide bonds of the structural/functional proteins.

According to a third aspect of the invention, there is provided, as an anti-atherogenic agent, the combination of (i) a substance or composition capable of lowering, in human plasma, the concentration of free sulfhydryl groups forming part of homocysteine and/or cysteine molecules, and (ii) a substance or composition capable of inhibiting the deleterious disulfide exchange reaction of homocysteine and/or cysteine with structural/functional proteins in arterial walls, thereby inhibiting directly the formation of atherosclerotic plaques in the human body.

The Applicant has found that the amino acid homocysteine [$\text{H}_2\text{NCH}(\text{COOH})\text{CH}_2\text{CH}_2\text{SH}$], and to a lesser extent, the amino acid cysteine [$\text{H}_2\text{NCH}(\text{COOH})\text{CH}_2\text{SH}$], are atherogenic agents in the human body, and that their atherogenic capability resides in their free sulfhydryl [-SH] groups. These molecules exert their atherogenic capability via the mechanism known as a disulfide exchange reaction, viz.



where R-SH = homocysteine and/or cysteine.

Furthermore, the Applicant has found that: (a) the reaction is critically dependent on the amount or degree of mechanical and/or chemical stress that is exerted on the relevant glycoprotein(s) and (b) that the reaction is more specific (irreversible) for homocysteine than for cysteine.

Hence, a substance or composition which lowers the concentration of the free sulfhydryl group of homocysteine and/or cysteine will act as an anti-atherogenic agent capable of inhibiting directly atherosclerotic plaque formation in the human body. This lowering of the homocysteine and/or cysteine free sulfhydryl group concentration in the plasma can be effected either by the substance or composition reacting with, or causing a reaction of, the free sulfhydryl group, thereby converting it into another group and hence lowering or hindering its activity, or by inducing reduction of free homocysteine and/or cysteine levels in plasma. These homocysteine and/or cysteine levels can be "normal" levels, ie those of healthy people, or elevated levels, ie those of homocystinuric people or animals.

The substance or composition may hence be, or include, at least one oxidizing agent and/or an oxidizing promotion/catalyzing agent, and must as such be of optimal synergistical composition, so as to include, for example, at least one cupric and/or zinc salt; at least one vitamin such as vitamin B₂ (riboflavin), niacin (nicotinamide), dehydroascorbic acid and/or an appropriate derivative of these vitamins; at least one compound from the group of quinones or quinone derivatives, such as ubiquinone, vitamin K, or vitamin K analogues like menadione; at least one compound from the general broad group of substances known as bioflavonoids (or flavonoids) such as rutin, hesperidin and troxerutin; and/or at least one appropriate component or derivative of the group of steroid hormones such as estradiol or dehydro-epiandrosteron (DHEA) and its sulfate ester, dehydro-epiandrosterone sulfate (DHEAS), the latter two being major secretory products of the human adrenal gland.

Instead, or additionally, the substance or composition can be, or include, at least one disulfide compound of the formula X-S-S-Y where X-SH and Y-SH are the same or different and each is a sulfhydryl containing compound, and may be administered in the form X-SH alone, Y-SH alone, or as X-SH plus Y-SH

to form X-S-S-Y within the body by oxidation, or the substance may be administered initially in the form X-S-S-Y. Each of the three forms, viz X-SH, Y-SH and X-S-S-Y is capable of reacting with homocysteine (or cysteine) in the body (or plasma) to form, for example, mixed disulfides of the formula $H_2NCH(COOH)-CH_2CH_2-S-S-X$ and/or $H_2NCH(COOH)CH_2CH_2-S-S-Y$. Suitable sulfhydryl and/or disulfide containing compounds are believed to include cystamine, cysteamine, allyl-propyl disulfide, diallyl disulfide, glutathione (oxidized or reduced) and even cyst(e)ine, or the like.

Instead, or additionally, the substance or composition may be, or include, at least one compound capable of decreasing homocysteine and/or cysteine levels per se in plasma, by its function as a co-factor or co-substrate in the transsulfuration pathway or methionine metabolism. Examples of such compounds are Vitamin B₆, Vitamin B₁₂, folic acid, co-substrates such as betaine and choline, or their metabolic precursors.

Still further, the substance or composition may be, or include, salts like magnesium chloride and/or at least one compound from the group of substances known as bioflavonoids (or flavonoids) like hydroxyethyl-rutoside and/or at least one compound from the group of substances that have a vasodilatory or anti-vasoactive action, or for this matter, arterial cell relaxation action, for example, a substance like cilazapril, an angiotensin converting enzyme (ACE) inhibitor, ie all of them substances that are believed to react directly or indirectly with relevant arterial wall cells and/or relevant structural/functional proteins of the arterial wall cell-cement, so as to effectively reduce directly or indirectly the mechanical and/or chemical stress on susceptible disulfide bonds of the relevant structural/functional proteins like fibronectin in the arterial wall cell-cement, thereby protecting them from the deleterious disulfide exchange reactions with homocysteine and/or cysteine, as described above.

The substance or composition may be administered on its own, or in admixture with a pharmaceutically acceptable adjunct, and can typically be administered orally, in which case it can be in the form of a tablet including a suitable binder or carrier, capsule, syrup including a suitable base, or the like.

It can be administered at any desired dosage rate, within the so-called 'therapeutic window' range. For example, it can be administered at relatively low dosages to healthy persons having "normal" homocysteine and/or cysteine levels, eg prophylactic or preventative dosages, or at relatively high therapeutic dosages to homocystinuric persons. For example, folic acid can be administered at a daily dosage rate of about 5 milligram ("mgm"), pyridoxine hydrochloride (Vitamin B₆) at a daily rate of about 50 mgm, cyanocobalamin (Vitamin B₁₂) at a daily rate of about 0,05 mgm, and choline or betaine at a daily rate of about 5 gm.

According to a fourth aspect of the invention, there is provided the use of an active substance or composition capable of lowering, in plasma, the concentration of free sulfhydryl groups forming part of homocysteine and/or cysteine molecules, thereby to inhibit directly atherosclerosis in the human body, for the manufacturing of a pharmaceutical to inhibit atherosclerosis.

According to a fifth aspect of the invention, there is provided the use of an active substance or composition capable of inhibiting, ie hindering or lowering, the deleterious disulfide exchange reaction of homocysteine and/or cysteine with structural/functional proteins in the arterial wall, thereby directly inhibiting atherosclerosis in the human body, for the manufacturing of a pharmaceutical to inhibit atherosclerosis.

The invention extends also to the use of the combination of (i) an active substance or composition capable of lowering, in plasma, the concentration of free sulfhydryl groups forming part of homocysteine and/or cysteine molecules, and (ii) an active substance or composition capable of inhibiting the deleterious disulfide exchange reaction of homocysteine and/or cysteine with structural/functional proteins in the arterial wall, thereby directly inhibiting atherosclerosis in the human body, for the manufacturing of a pharmaceutical to inhibit atherosclerosis.

According to a sixth aspect of the invention, there is provided the use of a substance or composition capable of lowering, in plasma, the concentration of free sulfhydryl groups forming part of homocysteine and/or cysteine molecules, to inhibit atherosclerosis in the human body.

According to a seventh aspect of the invention, there is provided the use of a substance or composition capable of inhibiting, ie hindering or lowering, the deleterious disulfide exchange reaction of homocysteine and/or cysteine with structural/functional proteins in the arterial wall, to inhibit atherosclerosis in the human body.

The invention extends also to the use of both, or the combination of, said substance or composition capable of lowering the free sulfhydryl groups, and said substance or composition capable of inhibiting the deleterious disulfide exchange reaction, to inhibit atherosclerosis in the human body.

According to a eighth aspect of the invention, there is provided a method of inhibiting atherosclerosis in a human body, which comprises administering to the body an effective amount of a substance or composition capable of lowering the concentration of free sulfhydryl groups forming part of homocysteine and/or cysteine molecules in the body plasma, thereby to inhibit directly atherosclerosis in the body.

According to a ninth aspect of the invention, there is provided a method of inhibiting atherosclerosis in

an human body which comprises administering to the body an effective amount of a substance or composition capable of inhibiting the deleterious disulfide exchange reaction of homocysteine and/or cysteine with structural/functional proteins in the arterial wall, thereby inhibiting directly atherosclerosis in the body.

The invention extends also to the use of both, ie the combination of, said substance or composition capable of lowering the concentration of free sulfhydryl groups, and said substance capable of inhibiting the deleterious disulfide exchange reaction, in the method of atherosclerosis inhibition.

The method may also comprise monitoring plasma total homocysteine, homocysteine-cysteine mixed disulfide and/or cyst(e)ine levels, for example, by means of an amino acid analyser, using among others a procedure based on that set out under the heading 'ANALYTICAL PROCEDURE' hereunder. The method may further comprise administering sufficient of the substance or composition to control the body plasma homocysteine or cysteine level at a desired value. For example, by taking the normal fasting circulation plasma homocysteine-cysteine mixed disulfide level (X-Dis) as a marker, the free plasma homocysteine level must be controlled to such an extent, that the fasting concentration of this marker (X-Dis) is reduced to reach ideally a value $<1\mu\text{mol/L}$ plasma [the normal concentration of this marker (X-Dis) is usually between 1 and $7\mu\text{mol/L}$].

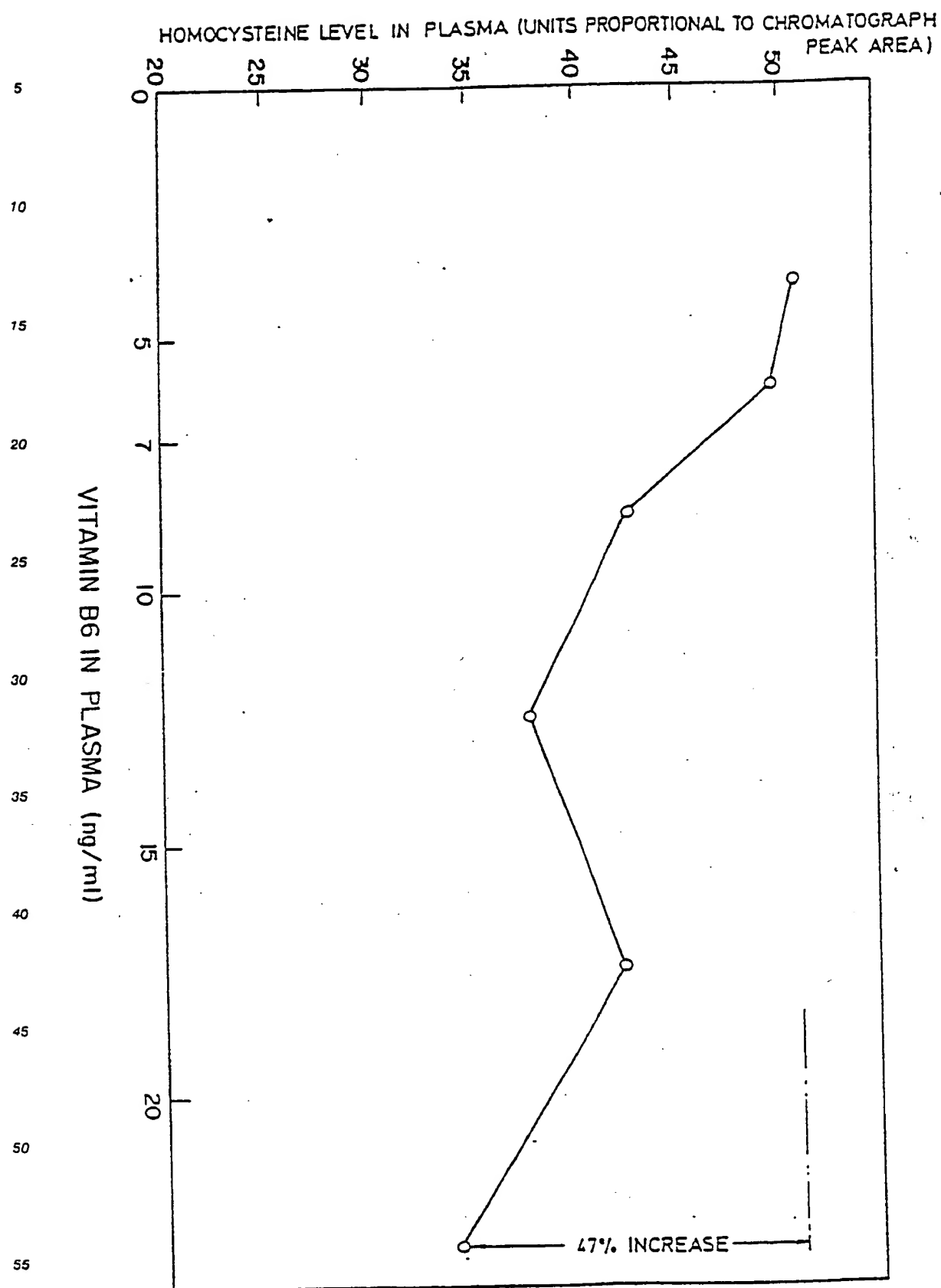
The method further comprises the administering of sufficient of the substance or composition to control the deleterious disulfide exchange reaction of homocysteine and/or cysteine with structural/functional proteins in the arterial wall. This deleterious disulfide exchange reaction can be evaluated and/or monitored by the following non-limiting procedure or example:

A long-term moderate homocystinemia (slightly elevated plasma homocysteine levels) can be induced in appropriate experimental animals by parental administration of methionine. The particular substance or composition may then be administered at a selected or comparative dosage rate for an appropriate period of time in these animals with elevated plasma homocysteine levels. The animals may then be killed at the end of the experiment and an appropriate segment of the aorta removed and investigated for a beneficial difference in endothelial permeability by light and/or scanning electron microscopy as compared to an appropriate control segment from animals which have received no such protective substance or composition. In this fashion, an appropriate dosage rate for dosing the human body with that substance can be determined.

Finally, the method may also comprise the optimizing of the inhibition of atherosclerosis in the human body through the use of a synergistical substance or composition for an appropriate period of time. For example, a substance or composition, like some of the vitamins listed hereinbefore, may be administered to the human body during its whole lifespan, ie from infant life until old age, so as to maintain the body plasma homocysteine at a desired value, and may additionally be fortified with a substance or composition that is capable of hindering or lowering the deleterious disulfide exchange reaction of homocysteine and/or cysteine with structural/functional proteins in the arterial wall, as and/or when needed.

The invention will now be described by way of example with reference to the following non-limiting test.

Vitamin B₆ (pyridoxal phosphate) levels of about 330 subjects were determined by means of a high pressure liquid chromatographic method. The subjects were grouped into categories according to their plasma B₆ reading, viz $<5\text{ ng B}_6/\text{ml}$, $5-7\text{ ng/ml}$, $7-10\text{ ng/ml}$, $10-15\text{ ng/ml}$, $15-20\text{ ng/ml}$, and $>20\text{ ng/ml}$. The $<5\text{ ng/ml}$ group contained subjects known to have low or subclinical B₆ levels, while the $>20\text{ ng/ml}$ group were identified as having been supplemented with B₆, eg in medication. For each group, the total plasma homocysteine levels of a randomized subgroup (10 or more subjects) were determined with an amino acid analyzer (using a procedure based on that set out under the heading "ANALYTICAL PROCEDURE" hereunder) and the mean or average calculated for each group. These values were plotted against the groups as indicated in the attached graph (see page 13).



From this graph it can clearly be seen that an approximately 4-fold increase in B₆ level leads to almost a 50% reduction in homocysteine levels, hence confirming that by administering a substance in accordance with the invention, such as Vitamin B₆, homocysteine levels are lowered. When these lowered levels are maintained for a long period of time (for example life-long), this may lead to a concomitant degree of inhibition of atherosclerosis, ie reduction in ischaemic diseases, as a result of the following mechanism identified by the Applicant: at low or "normal" homocysteine levels in the plasma, there is normally little or only a low rate of atherosclerotic plaque formation of endothelial permeability damage to the arterial wall. However, at elevated homocysteine levels, as would be evidenced in homocystinuric patients, atherosclerosis takes place at an accelerated rate as a result of disulphide exchange of susceptible bonds in cell-to-cell or cell-to-substrate adhesive proteins, and/or to cell membrane proteins and/or to cell connective tissue proteins due to the -SH group of homocysteine, resulting in, amongst others, loss of the structural/functional properties of the proteins concerned, for example, fibronectin and/or proteoglycans, with an concomitant change or damage to the endothelial permeability, or for that matter, the endothelial cell integrity of the arterial wall and/or other arterial wall cells and which may eventually lead to, amongst other pathological changes, endothelial denudation or desquamation of the arterial wall. Furthermore, even in persons having "normal" homocysteine levels, when the arterial cells are under additional mechanical and/or chemical stress, eg as normally happens in arteries in proximity to the heart, such as the coronary arteries, due to, amongst others, rheological factors, and/or in smokers, and/or in persons with high blood pressure, high total serum cholesterol levels and/or other forms of stress such as are induced by inflammation and/or vasoactive substances, or such as do occur in type A personalities, "normal" homocysteine levels also initiate atherosclerosis by the mechanism set out hereinbefore, with the resultant atherosclerosis also being enhanced by these other factors, or in relevant arteries.

The same applies, in essence, to cysteine, although according to a concentration criterion, only less efficiently.

An important aspect of the identified mechanism is (a) that the coronary artery is normally already continuously exposed to so-called "extra mechanical stress" due to pulsatile blood flow from the heart. According to the above-identified mechanism, this part of the artery (or adjacent parts) is more prone to occlusive atherosclerotic plaque development than the peripheral or cerebral arteries at normal plasma homocysteine levels; and (b) that according to the identified mechanism, homocysteine at 'normal' as well as elevated plasma homocysteine levels, leads to the most important initiation event in the aetiology of atherosclerosis, viz increased or damaged endothelial permeability. The difference between 'normal' and elevated plasma homocysteine levels, is not only related to a difference in the rate of endothelial permeability damage, but also to the character of ensuing atherosclerotic plaques, viz mainly ischaemic heart disease at 'normal' levels and essentially occlusive peripheral and cerebral arterial diseases, as well as thrombophlebitis at moderate to highly elevated plasma homocysteine levels. Additionally, when the abovementioned mechanical and/or chemical stress factors are increased at 'normal' plasma homocysteine levels, the ensuing occlusive complications may be both in the heart, peripheral and cerebral arteries.

Hence, by controlling homocysteine free sulfhydryl group levels, and/or the deleterious disulfide exchange reaction of homocysteine and/or cysteine the Applicant believes that atherosclerosis can be controlled or inhibited.

ANALYTICAL PROCEDURE

Method: 1) The plasma was prepared according to a standard procedure (ie blood cooled immediately after venepuncture and plasma separated within 1 hour). The plasma was then conveniently stored frozen for any length of time, if necessary.

2) Homocysteine was released from the plasma by dithiotreitol treatment and deproteinized with sulfosalicylic acid. After an adjustment of the pH, the sample was analyzed directly. According to the literature however, it is actually this step that must conventionally be avoided (due to instability) and homocysteine must first be derivatized before analysis (to the S-carboxymethyl derivativ for example). Apart from being laborious, the latter step however also leads to losses in yield.

Direct analysis were however performed under the following precautionary conditions: a) Due to low concentrations and poor colour yield with ninhydrin, homocysteine must be separated quite well from all contaminating substances. For this purpose the analyzer must have excellent resolving capabilities, high sensitivity, as well as an excellent on-line chromatography data system. b) Losses do occur however on the column, especially in the plasma range from 1 to 5 umol/L. This was however compensated for by the use

of a carefully constructed standard curve. A special short program for the separation of homocysteine was developed, with plasma homocysteine yields that are in good agreement with the capillary gas chromatography-mass spectroscopy technique developed recently.

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Claims

1. As an anti-atherogenic agent, a substance or composition capable of lowering, in human plasma, the concentration of free sulfhydryl groups forming part of homocysteine and/or cysteine molecules, thereby
10 inhibiting directly the formation of atherosclerotic plaques in the human body.
2. An anti-atherogenic agent according to Claim 1, characterized in that the substance or composition comprises at least one oxidizing agent and/or an oxidizing promotion/catalyzing agent.
3. An anti-atherogenic agent according to Claim 2, characterized in that the substance or compound is selected from a cupric salt, a zinc salt, vitamin B₂, niacin, dihydroascorbic acid, quinone, a quinone
15 derivative, a bioflavonoid compound, hesperidin, a steroid hormone, and a steroid hormone derivative.
4. An anti-atherogenic agent according to Claim 1, characterized in that the substance or composition comprises at least one disulfide compound of the formula X-S-S-Y where X-SH and Y-SH are the same or different and are each a sulfhydryl containing compound, with X-S-S-Y being capable of reacting with homocysteine and/or cysteine in the human body to form mixed disulfides.
- 20 5. An anti-atherogenic agent according to Claim 1, characterized in that the substance or composition comprises a compound X-SH and/or Y-SH, where X and Y are the same or different, and are capable of forming the disulfide compound X-S-S-Y within the body, with X-SH, Y-SH and X-S-S-Y being capable of reacting with homocysteine and/or cysteine in the human body to form mixed disulfides.
6. An anti-atherogenic agent according to Claim 1, characterized in that the substance or composition
25 comprises at least one compound capable of decreasing homocysteine and/or cysteine levels *per se* in plasma, by its function as a co-factor or co-substrate in the transsulfuration pathway of methionine metabolism.
7. An anti-atherogenic agent according to Claim 6, characterized in that the compound is selected from Vitamin B₆, Vitamin B₁₂, folic acid, betaine, choline, or their metabolic precursors.
- 30 8. As an anti-atherogenic agent, a substance or composition capable of inhibiting the deleterious disulfide exchange reaction of homocysteine and/or cysteine with structural/functional proteins in arterial walls, thereby inhibiting directly the formation of atherosclerotic plaques in the human body.
9. An anti-atherogenic agent according to Claim 8, characterized in that the substance or composition comprises at least one compound capable of reacting directly or indirectly with the arterial wall cells and/or
35 with the structural/functional proteins of the arterial wall cell-cement, thereby to reduce directly or indirectly the mechanical and/or chemical stress on susceptible disulfide bonds of the structural/functional proteins.
10. An anti-atherogenic agent according to Claim 9, characterized in that the compound is selected from magnesium chloride, a bioflavonoid, a vasodilator, and an arterial cell relaxation agent.
11. As an anti-atherogenic agent, the combination of (i) a substance or composition capable of lowering,
40 in human plasma, the concentration of free sulfhydryl groups forming part of homocysteine and/or cysteine molecules, and (ii) a substance or composition capable of inhibiting the deleterious disulfide exchange reaction of homocysteine and/or cysteine with structural/functional proteins in arterial walls, thereby to inhibit directly the formation of atherosclerotic plaques in the human body.
12. The use of a substance or composition capable of lowering, in body plasma, the concentration of
45 free sulfhydryl groups forming part of homocysteine and/or cysteine molecules, thereby to inhibit directly atherosclerosis in the human body, for the manufacture of a pharmaceutical to inhibit atherosclerosis.
13. The use of a substance or composition capable of inhibiting the deleterious disulfide exchange reaction of homocysteine and/or cysteine with structural/functional proteins in the arterial wall, thereby inhibiting directly atherosclerosis in the human body, for the manufacture of a pharmaceutical to inhibit
50 atherosclerosis.
14. The use of a substance or composition capable of lowering, in body plasma, the concentration of free sulfhydryl groups forming part of homocysteine and/or cysteine molecules, to inhibit atherosclerosis in the human body.
15. The use of a substance or composition capable of inhibiting the deleterious disulfide exchange
55 reaction of homocysteine and/or cysteine with structural/functional proteins in arterial walls, to inhibit atherosclerosis in the human body.

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(54)

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PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention
shall be considered, for the purposes of subsequent
proceedings, as the European search report

Application Number

EP 89 11 1246

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 4)
X	EUROPEAN JOURNAL OF CLINICAL INVESTIGATION, vol. 8, no. 4, August 1978, page 199, Blackwell Scientific Publications; L.A. HARKER et al.: "Sulphydryl-mediated vascular disease" * The whole document *	1,2,6,8 9,12-15	A 61 K 33/34 A 61 K 33/30 A 61 K 31/525 A 61 K 31/455 A 61 K 31/375 A 61 K 31/105
X	NATURE, vol. 231, no. 5302, 11th June 1971, pages 391-392; K.S. MCCULLY et al.: "Homocysteine metabolism in scurvy, growth and arteriosclerosis" * The whole document *	1,26,8, 9,12-15	A 61 K 31/095 A 61 K 31/68 A 61 K 31/505 A 61 K 31/205 A 61 K 31/00
			TECHNICAL FIELDS SEARCHED (Int. Cl. 4)
			A 61 K
INCOMPLETE SEARCH			
<p>The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims</p> <p>Claims searched completely : Claims searched incompletely : 1-3,6,8,9,12-15 Claims not searched : Reason for the limitation of the search:</p> <p>See sheet -C-</p>			
Place of search		Date of completion of the search	Examiner
THE HAGUE		12-09-1991	GAC G.
CATEGORY OF CITED DOCUMENTS			
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons A : member of the same patent family, corresponding document	

EPO FORM 1503 03/82 (P0407)



CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

- ☐ All claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for all claims.
- ☐ Only part of the claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid,
namely claims:
- ☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions,
namely:

See sheet -B-

- ☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.
- ☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid,
namely claims:
- ☐ None of the further search fees has been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims,
namely claims: 1,2,3(partially),6,8,9,12-15



European Patent
Office

PARTIAL EUROPEAN SEARCH REPORT

Page 2

Application Number

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DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. Cl. 4)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
X	THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol 262, no. 21, 25th July 1987, pages 10098-10103, The American Society of Biological Chemists, Inc.; J. W. HEINECKE et al.: "The role of sulfur-containing amino acids in superoxide production and modification of low density lipoprotein by arterial smooth muscle cells" * The whole document *	1,2,6,8 ,9,12-15	
X	PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, vol. 121, no. 2, February 1966, pages 452-455; S. RENAUD: "Prevention by methionine feeding of arteriosclerosis and thrombosis in hyperlipemic rats" * The whole document *	1,2,6,8 ,9,12-15	
X	PATENT ABSTRACTS OF JAPAN, vol. 7, no. 46, (C-153)[1191], 23rd February 1983; & JP-A-57 197 284 (EIICHI SUENAGA) 03-12-1982 * The whole document *	1,2,3,6 ,8,9,12-15	TECHNICAL FIELDS SEARCHED (Int. Cl. 4)
A	THE JOURNAL OF CLINICAL INVESTIGATION, vol. 77, no. 4, April 1986, pages 1370-1376, The American Society for Clinical Investigation, Inc.; G. STARKEBAUM et al.: "Endothelial cell injury due to copper-catalyzed hydrogen peroxide generation from homocysteine" * The whole document *	1,2,3,6 ,8,9,12-15	

EPO FORM 1501 (01.87) (Pw10)



LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions,

namely:

In view of the following documents: "Eur. J. Clin. Invest. 1978: HARKER et al.; Nature 1971, McCully et al.; J. Biol. Chem. 1987, Heinecke et al.; and Proc. Soc. Exp. Biol. Med. 1966, Renaud", which describe the general common inventive concept and especially in view of JP-A-57 197 284 which describes the use of cupric salts in oxidative processes and for treating atherosclerosis, the general inventive concept of claims 1 and 2 linking the compounds is no longer valid. Therefore the application contains the various following subjects:

1. Claims 1,2,3(partially),6,8,9,12-15: Use of a substance or composition capable of lowering the concentration of free sulfhydryl groups of homocysteine/cysteine or decreasing the levels of homocysteine/cysteine per se, or reacting with the functional or structural proteins of the arterial wall cells, in the manufacture of a medicament for treating atherosclerosis.
2. Claim 3(partially): Use of a zinc salt in the manufacture of a medicament for treating atherosclerosis.
3. Claims 3,7(partially): Use of vitamine B2, B6 or B12, niacin, folic acid or dihydroascorbic acid in the manufacture of a medicament for treating atherosclerosis.
4. Claim 3(partially): Use of a quinone or a derivative thereof in the manufacture of a medicament for treating atherosclerosis.
5. Claims 3,10(all partially): Use of a bioflavonoid, especially hesperidin in the manufacture of a medicament for treating atherosclerosis.
6. Claim 3(partially): Use of a steroid hormone or a derivative thereof in the manufacture of a medicament for treating atherosclerosis.
7. Claim 4: Use of a substance or composition comprising at least one disulfide compound of the formula X-S-S-Y wherein X and Y may be the same or different and may contain sulfhydryl groups, capable or reacting with homocysteine and/or cysteine in the human body to form mixed disulfides, in the manufacture of a medicament for treating atherosclerosis.

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8. Claim 5: Use of a substance or composition comprising a compound SH-X or/and Y-SH wherein X and Y are the same or different, capable of forming a disulfide compound X-S-S-Y within the body, with X-SH, Y-SH and X-S-S-Y being capable of reacting with homocysteine and/or cysteine in the human body to form mixed disulfides, in the manufacture of a medicament for treating atherosclerosis.

9. Claim 7(partially): Use of betaine or its metabolic precursors in the manufacture of a medicament for treating atherosclerosis.

10. Claim 7(partially): Use of choline or its metabolic precursors in the manufacture of a medicament for treating atherosclerosis.

11. Claim 10(partially): Use of magnesium chloride in the manufacture of a medicament for treating atherosclerosis.

12. Claim 10(partially): Use of a vasodilator in the manufacture of a medicament for treating atherosclerosis.

13. Claim 10(partially): Use of an arterial cell relaxation agent in the manufacture of a medicament for treating atherosclerosis.

14. Claim 11: Use of the combination of 1) a substance or composition capable of lowering, in human plasma, the concentration of free sulfhydryl groups forming part of homocysteine and/or cysteine molecules and 2) a substance or composition capable of inhibiting the deleterious disulfide exchange reaction of homocysteine and/or cysteine with structural/functional proteins in arterial walls, thereby inhibiting directly the formation of atherosclerotic plaques in the human body in the manufacture of a medicament for treating atherosclerosis.



Obscurities

Claims searched incompletely: 1-3,6,8,9,12-15:

1. In accordance with the EP Guidelines for the searching division Chap. III, par. 3.6 (part B), the search has covered the subject matter to which the claims might reasonably be expected to be directed after they have been amended, namely: "The use of a substance or composition capable of a) lowering in human plasma, the concentration of free sulfhydryl groups forming part of homocysteine and/or cysteine molecules, thereby inhibiting directly the formation of atherosclerotic plaque in the human body, or b) decreasing homocysteine and/or cysteine levels per se in plasma, by its function as a co-factor or co-substrate in the transsulfuration pathway of methionine metabolism, or c) inhibiting the deleterious disulfide exchange reaction of homocysteine and/or cysteine with structural/functional proteins in arterial wall, thereby inhibiting directly the formation of atherosclerotic plaques in the human body or reducing directly or indirectly the mechanical and/or chemical stress on susceptible disulfide bonds on the functional/structural proteins, in the manufacture of a medicament for treating atherosclerosis".
2. Neither a pharmacological mechanism (e.g. interaction with certain internal molecules (e.g. homocysteine/cysteine) or proteins (of the arterial wall)), nor a chemical reactivity (type of reaction taking place in situ)(or co-factor of an enzymatic reaction for formation of disulfide bonds) can sufficiently describe the chemical structure of the formulas meant in the claims. The broadness and the vagueness concerning the compounds does not allow a meaningful search.

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